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Stimulation of Synthesis of Free Chondroitin Sulfate Chains by β-D-Xylosides in Cultured Cells

(glycosaminoglycans/cartilage cells/mesenchyme cells/glial cells/hepatoma)

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ABSTRACT **Previous studies have shown that D-xylose partially overcomes the puromycin inhibition of chondroitin sulfate synthesis in cultured chick embryo chondrocytes. Likewise, D-xylose stimulates chondroitin sulfate synthesis by limb bud mesenchyme cells previously treated with BrdU or limb bud cartilage cells treated with puromycin. The studies reported here show that *p*-nitrophenyl-β-D-xylopyranoside and 4-methyl-umbelliferyl-β-D-xylopyranoside cause a much greater stimulation than does D-xylose and are active at much lower concentrations. In contrast to D-xylose, the xylosides strikingly stimulate chondroitin sulfate synthesis in predifferentiated mesenchyme cells. The xylosides stimulate synthesis of chondroitin sulfate by rat glial cell tumor cells (RC-6), a mouse neuroblastoma (C1300, NB41A), and two strains of cultured rat hepatoma cells (HTC, H_4). These results indicate that certain types of nonconnective tissue cells contain the enzymic machinery for synthesis of chondroitin sulfate which is normally not utilized because of limited synthesis of core protein and/or xylosyltransferase. The β-xylosides may be used as a probe of the capacity of various cell types to synthesize sulfated glycosaminoglycans.**

Sulfated glycosaminoglycans in animal tissues are bound covalently to protein by way of a galactosyl-galactosyl-xylosyl linkage to the hydroxyl groups of serine (1). Biosynthesis of these proteoglycans requires formation of core protein followed by stepwise addition of the individual monosaccharides from uridine diphosphosugars by a series of glycosyltransferases (2–5). Presumably the same enzymes catalyze the transfer of the individual monosaccharides to acceptors *in vitro* (3–6).

In minced cartilage, Brett and Robinson (7) showed that high concentrations of D-xylose reversed the inhibition by puromycin of chondroitin sulfate synthesis. Most likely, xylose acted as an initiator of polysaccharide chain formation, circumventing the requirement for core protein and xylosyltransferase. These results were confirmed and extended in the studies of Levitt and Dorfman (8), who additionally showed that high concentrations of D-xylose increased chondroitin sulfate synthesis in progeny of BrdU-treated limb bud cells.

More recently, Okayama *et al.* (9) observed that *p*-nitrophenyl-β-D-xyloside acted as an exogenous substrate for the cell-free particulate galactosyltransferase system. Furthermore, this compound stimulated $^{35}SO_4$ incorporation into material characterized as protein-free chondroitin sulfate chains in puromycin-treated cartilage slices (9). The present report is concerned with the stimulation of chondroitin sulfate synthesis by β-D-xylopyranosides in cultures of chondrocytes and limb bud cells treated with BrdU and puromycin. In addition, enhancement of chondroitin sulfate synthesis by β-D-xylopyranosides was demonstrated in four other cell types that normally synthesize only minimal amounts of this sulfated glycosaminoglycan.

MATERIALS AND METHODS

Materials. F-12 powdered medium was obtained from North American Biologicals, Inc. Modified Eagle's medium (10), fetal calf serum, horse serum, 2.5% lyophilized trypsin, 0.25% (w/v) trypsin–EDTA solution, and Hank's balanced salt solution (calcium- and magnesium-free) were purchased from Grand Island Biologicals Co. D-Xylose was obtained from Pfanstiehl; *p*-nitrophenyl-β-D-xylopyranoside, *p*-nitrophenyl-α-D-xylopyranoside, and 4-methylumbelliferyl-β-D-xylopyranoside from Koch-Light Laboratories, Ltd., 4-methylumbelliferyl-β-D-galactopyranoside from Pierce Chemical Co., *p*-nitrophenyl-β-D-galactopyranoside, *p*-nitrophenyl-α-D-galactopyranoside, and *p*-nitrophenyl-*N*-acetyl-β-D-galactosamine from Sigma Chemical Co. Puromycin dihydrochloride was purchased from Nutritional Biochemicals Co. and 5-bromo-2′-deoxyuridine (BrdU) from Sigma. The radioactive precursors, $H_2{}^{35}SO_4$ (carrier-free, 43 Ci/mg) and sodium [*U*-^{3}H]acetate (100 Ci/mol), were obtained from New England Nuclear Corp. Chondroitinase ABC, prepared by Seikagaku Kogyo Co., Ltd., was purchased from Miles Laboratories, Inc. Streptococcal hyaluronidase (Varidase) was obtained from Lederle Laboratories. Carrier chondroitin-4-sulfate was donated by Dr. J. A. Cifonelli, and twice-crystallized papain was a gift from Dr. Lennart Rodén.

Cell Culture. Cultures of chondrocytes from sternal cartilage of 15-day chick embryos were established according to procedures described by Cahn *et al.* (11). Cells were plated at an initial density of 2 to 5 × 10^5 cells on 60-mm Falcon tissue culture dishes in F-12 medium with 7% horse serum and 10% fetal calf serum (v/v). After the cells were allowed to attach to the dishes (about 3 days), subsequent growth was maintained by feeding the cells at 2-day intervals with modified Eagle's medium containing 7% horse serum and 10% fetal calf serum. When appropriate, BrdU was added (32 μM) from the sixth to the tenth day of culture after plating.

Embryonic chick limb buds were cultured as described (12). Briefly, limb buds (stage 23-24) were dissociated in 0.25% trypsin–EDTA and 2.5 × 10^7 cells were plated on 60-mm plastic culture dishes in F-12 medium with 7% horse serum and 8% fetal calf serum. Cultures either remained untreated or were exposed to 32 μM BrdU for the first 2 days of growth. Subsequently, the BrdU was removed and cells were maintained for an additional 7 days in medium without BrdU. Cells were fed every other day.

Rat glial cells (clonal strain C-6) and mouse neuroblastoma (C1300 clonal strain NB41A) were a gift of Dr. Gordon Sato. Rat hepatoma cells were obtained from Drs. D. Haggerty and G. Popjak. Trypsinized suspensions of rat glial cells were plated at a density of 5×10^5 cells per 100-mm plastic tissue culture dish. During the following 7 days, cells were fed twice with Eagle's medium supplemented with 10% fetal calf serum. Neuroblastoma cells and hepatoma cells were cultured under the same conditions.

Glycosaminoglycan Synthesis. For assay of sulfated glycosaminoglycan synthesis, the xylosides and either 5 μCi/ml of $H_2{}^{35}SO_4$ or 10 μCi/ml of [^{3}H]acetate were added for 6 hr on the last day of culture. Media and cells were collected, and the amount of radioactive glycosaminoglycan was determined as described (13) after addition of 2 mg of carrier chondroitin-4-sulfate.

Chondroitinase Treatment. Chondroitinase ABC digestions were performed as described by Saito *et al.* (14) in 10 mM Tris-acetate buffer (pH 8.0) for 21 hr at 37° with 0.2–0.4 units of enzyme in 0.8 ml of incubation mixture.

Streptococcal Hyaluronidase. Digestion mixtures containing 0.15 mg of Lederle Varidase in a total volume of 1.15 ml of 10 mM phosphate buffer (pH 6.0) and 0.15 M NaCl (15) were incubated for 18 hr at 37°.

Nitrous Acid Degradation. Samples were treated with 1 volume of 5% sodium nitrite and 1 volume of 33% acetic acid at room temperature for 2 hr according to the procedure of Dische and Borenfreund (16). The mixtures were then lyophilized and dissolved in distilled water.

Gel Filtration. Labeled polysaccharide was chromatographed on columns of Sephadex G-50 (0.8 × 100 cm) previously calibrated with bovine serum albumin and UDP-*N*-acetylgalactosamine. Samples were eluted with 0.2 M NaCl in fractions of 0.8 ml, and aliquots (0.1 or 0.25 ml) of each fraction were assayed for radioactivity.

Molecular size estimates of chondroitin sulfate chains produced by limb bud cells treated with 4-methylumbelliferyl-β-D-xyloside are based on elution behavior on a calibrated Sephadex G-200 column (1 × 100 cm) according to Hopwood and Robinson (17). Samples were eluted with 0.2 M NaCl in fractions of 5 ml, and aliquots (0.5 ml) of each fraction were assayed for radioactivity. A coincident peak of fluorescence after hydrolysis was obtained (unpublished observations), indicating that these polysaccharide chains contain the xyloside at the reducing terminus.

RESULTS

Effect of β-D-Xylosides on Chondroitin Sulfate Synthesis by Chondrocytes. Growth of chondrocytes in the presence of BrdU results in a marked reduction in chondroitin sulfate synthesis (18–20). The results displayed in Table 1 indicate that addition of *p*-nitrophenyl-β-D-xyloside and 4-methylumbelliferyl-β-D-xyloside to differentiated chondrocytes previously grown in the presence of BrdU stimulates sulfate incorporation into glycosaminoglycans to levels approximately equal to those of cells cultured in the absence of the drug. Similar stimulatory effects by the β-xylosides are observed when cultures are treated with puromycin, which previously was shown to inhibit sulfated glycosaminoglycan synthesis by cartilage (22). Chondrocytes treated with both BrdU and puromycin are likewise stimulated by the β-xylosides to produce high levels of glycosaminoglycans.

TABLE 1. *Specificity of effect of xylosides on glycosaminoglycan synthesis by chondrocytes**

Addition	Control	Puromycin	BrdU	BrdU + puromycin
Experiment I				
None	36,000	4,250	2,610	1,220
p-Nitrophenyl-β-D-xylose	56,500	53,700	44,900	39,000
p-Nitrophenyl-α-D-xylose	37,400	2,460	4,400	1,350
4-Methylumbelliferyl-β-D-xylose	56,300	N.D.	34,750	31,200
β-D-Xylose	42,400	15,000	21,200	17,700
Experiment II				
None	41,000	5,730	4,700	408
p-Nitrophenyl-β-D-xylose	94,400	102,000	68,600	38,300
p-Nitrophenyl-β-D-galactose	35,000	7,220	2,980	605
p-Nitrophenyl-α-D-galactose	35,800	1,430	4,400	553
4-Methylumbelliferyl-β-D-galactose	35,500	1,730	2,900	152
p-Nitrophenyl-β-D-*N*-acetylgalactosamine	47,600	2,060	2,470	785

On the tenth day of culture, chondrocytes (3×10^6 cells/plate) growing in the presence or absence of 32 μM BrdU were incubated with 5μ Ci/ml of $H_2{}^{35}SO_4$ plus the indicated compounds at a final concentration of 1 mM (except for D-xylose, which was used at a final concentration of 42 mM) and with or without 10 μg/ml of puromycin for 6 hr. Glycosaminoglycans were isolated from pooled cells plus media according to Dorfman and Ho (13). N.D., not determined.

* Results are expressed as cpm/10^6 cells.

Glycosaminoglycan synthesis in control cultures is also stimulated by β-xylosides. The magnitude of this effect varied among different experiments, indicating that certain cultures have a greater potential for chondroitin sulfate chain synthesis than is expressed; presumably core protein or xylosyltransferase is limiting. In these cases, *p*-nitrophenyl-β-D-xyloside stimulates sulfate incorporation so that control and puromycin-treated cells reach approximately the same levels (Table 1).

The effect of the β-xylosides on glycosaminoglycan synthesis by chondrocytes (Table 1) is specific for both the anomeric linkage and the glycone.

D-Xylose partially reverses the BrdU-induced inhibition of chondroitin sulfate synthesis in limb bud cells and chondrocytes (8, 23). Xylose stimulates sulfate incorporation in BrdU- and puromycin-treated chondrocytes to about 50% of the control level at an optimal concentration of 43 mM. This concentration of xylose is 40-fold greater than the concentration of the β-xylosides that cause maximal effects (Fig. 1). Stimulation is observed at concentrations of *p*-nitrophenyl-β-D-xyloside as low as 0.01 mM, but the optimal concentration is about 0.5 mM.

The effect on chondroitin sulfate synthesis by *p*-nitrophenyl-β-D-xyloside is rapid, so that in the presence of the compound, within 1–2 hr an appreciable increase in sulfate

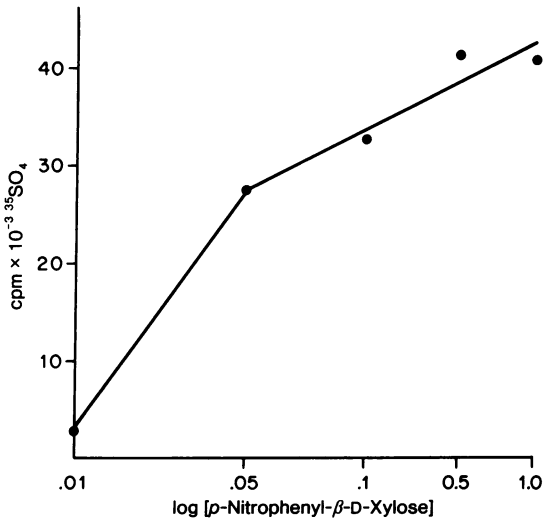

FIG. 1. Dose response of puromycin-treated chondrocytes to *p*-nitrophenyl-β-D-xyloside. On the tenth day of growth, chondrocytes (1.4×10^6 cells per plate) were incubated with 5 μCi/ml of $H_2{}^{35}SO_4$, 10 μg/ml of puromycin, and concentrations of *p*-nitrophenyl-β-D-xyloside ranging from 0.01 to 1.0 mM for 6 hr. Glycosaminoglycans were isolated from pooled cells plus media as described (13).

incorporation by both control and puromycin-treated chondrocyte cultures is observed. The maximum effect occurs at 3–6 hr. Sulfate incorporation into glycosaminoglycans by control and puromycin-treated cultures increases in a parallel fashion during continued incubation in the presence of the β-xylosides.

Effect of β-Xylosides on Chondroitin Sulfate Synthesis in Limb Bud Cultures. Earlier studies have shown that growth of limb bud mesenchyme in BrdU for 48 hr results in marked reduction of chondroitin sulfate synthesis even when measured 7 days after growth in BrdU-free medium (12). As shown with D-xylose, both *p*-nitrophenyl-β-D-xyloside and 4-methylumbelliferyl-β-D-xyloside stimulate sulfate incorporation into glycosaminoglycans in the progeny of BrdU-treated cultures (Table 2). The inhibition of chondroitin sulfate synthesis by puromycin in limb bud cells is also reversed by addition of the β-xylosides.

TABLE 2. *Effect of xylosides on chondroitin sulfate synthesis by limb bud cells (9-day cells)**

Addition	Control	Control + puromycin	BrdU-progeny	BrdU-progeny + puromycin
None	69,343	13,071	5,449	1,797
D-Xylose	103,154	43,363	22,625	13,196
p-Nitrophenyl-α-D-xylose	66,773	17,991	15,679	3,493
p-Nitrophenyl-β-D-xylose	208,458	135,615	72,821	48,663
4-Methylumbelliferyl-β-D-xylose	184,217	132,424	64,459	50,273

High density cultures of control and BrdU-treated limb bud cells were labeled with 5 μCi/ml of $H_2{}^{35}SO_4$ plus the indicated D-xyloside derivatives at concentrations of 1 mM, except D-xylose (21.2 mM), with or without 10 μg/ml of puromycin for 6 hr on the ninth day of culture. Glycosaminoglycans were isolated from pooled cells and media (13).

* Results are expressed as cpm/plate.

TABLE 3. *Effect of xylosides on chondroitin sulfate synthesis (chick limb bud mesenchyme)**

Addition	Control	Puromycin
None	17,238	789
D-Xylose	19,753	1,733
p-Nitrophenyl-β-D-xylose	81,232	30,539
4-Methylumbelliferyl-β-D-xylose	88,079	35,751

Chick limb bud mesenchyme cells after 1 day in culture were labeled with 5 μCi/ml of $H_2{}^{35}SO_4$ and treated with 1 mM concentration of the D-xyloside derivatives, except D-xylose (21.2 mM), with or without 10 μg/ml of puromycin for 6 hr. Cells plus media were pooled and glycosaminoglycans isolated as described (13).

* Results are expressed as cpm/plate.

Cultures of 1-day limb bud mesenchyme (before differentiation to cartilage) synthesize only 10% as much glycosaminoglycans as cells in culture for 9 days (which had differentiated). Chondroitin sulfate synthesis in these cells is stimulated by addition of β-xylosides (Table 3) in the absence (4-fold) and presence (40-fold) of puromycin. These results are consistent with the demonstrable glycosyltransferase activities in 1-day predifferentiated cells (23). Little or no stimulation of sulfate incorporation is observed by addition of D-xylose to 1-day cultures, in agreement with the previous report (23).

When papain-digested proteoglycan (13) from β-xyloside-stimulated, 1-day limb bud cultures that had been treated with puromycin was chromatographed over Sephadex G-200, the chain length distribution pattern was similar to that which has been reported for chondroitin sulfate chains from several sources (17).

Effect of β-Xylosides on Glycosaminoglycan Production in Noncartilaginous Cells. The demonstration of β-xyloside stimulation in predifferentiated, 1-day limb bud cells, which normally produce low levels of chondroitin sulfate, led to an examination of the effect of β-xylosides on other cell types. β-D-Xyloside or 4-methylumbelliferyl-β-D-xyloside stimulates glycosaminoglycan synthesis 2- to 10-fold in glial cells, neuroblastoma, and two strains of hepatoma cells (Table 4). In glial and neuroblastoma cultures, glycosaminoglycans were characterized by susceptibility to chondroitinase ABC, nitrous acid degradation, and streptococcal hyaluronidase.

Although [^{3}H]acetate and $H_2{}^{35}SO_4$ were included in the experiment shown in Table 5, the data and the calculations are based on [^{3}H]acetate incorporation so that hyaluronic acid synthesis could be quantitated. The greatest increase in radioactivity (10-fold) is observed in that fraction remaining after streptococcal hyaluronidase treatment, which is sensitive to chondroitinase ABC and, therefore, may contain a mixture of chondroitin 4/6-sulfate and dermatan sulfate. [Previous studies have shown that the contribution of dermatan sulfate in glial cells is minimal (13).] There was a more modest stimulation of heparan sulfate synthesis and some stimulation of hyaluronic acid synthesis. The mechanism of the latter is unexplained since this glycosaminoglycan is not known to contain the xyloside linkage. Further studies of this phenomenon are required. A substantial stimulation of glycosaminoglycan production by addition of 4-methylumbelliferyl-β-D-xyloside to cultures of normal, Marfan, and

TABLE 4. *Stimulation of glycosaminoglycan synthesis by xylosides*

	Total $^{35}SO_4$-glycosaminoglycans (cpm/10^6 cells)
Glial cells (RGC-6)	
Control	6,023
+ 4-Methylumbelliferyl-β-D-xylose	23,602
+ *p*-Nitrophenyl-β-D-xylose	24,090
Neuroblastoma (NB41A)	
Control	822
+ 4-Methylumbelliferyl-β-D-xylose	5,866
+ *p*-Nitrophenyl-β-D-xylose	7,748
Hepatoma (H_4)	
Control	703
+ 4-Methylumbelliferyl-β-D-xylose	2,809
Hepatoma (HTC)	
Control	2,128
+ 4-Methylumbelliferyl-β-D-xylose	5,053

Rat glial cells (3×10^7 cells per plate) and mouse neuroblastoma cells (1.1×10^7 cells per plate) were incubated with 5 μCi/ml of $H_2{}^{35}SO_4$ and 0.5 mM 4-methylumbelliferyl-β-D-xyloside or *p*-nitrophenyl-β-D-xyloside for 6 hr. Two strains of hepatoma cells (about 2×10^7 cells per plate each) were incubated with 5 μCi/ml of $H_2{}^{35}SO_4$ and 0.1 mM 4-methylumbelliferyl-β-D-xyloside for 6 hr. Glycosaminoglycans from pooled cells plus media were isolated (13).

TABLE 5. *Stimulation of glycosaminoglycan synthesis by xylosides in glial cells**

	Control	*p*-Nitrophenyl-β-D-xylose
Total glycosaminoglycans	7,883 (100%)	29,233 (100%)
Hyaluronic acid	2,782 (35%)	7,512 (26%)
Chondroitin 4/6-sulfate + dermatan sulfate	1,465 (19%)	13,067 (45%)
Heparan sulfate	1,631 (21%)	3,303 (11%)

Rat glial cells (2.4×10^7 cells per plate) were labeled with 5 μCi of $H_2{}^{35}SO_4$ and 10 μCi of [^{3}H]acetate in the presence or absence of 0.5 mM *p*-nitrophenyl-β-D-xyloside. (Data are presented as [^{3}H]acetate incorporation.) After isolation of the total glycosaminoglycans as described (13), the individual products were identified by sequential streptococcal hyaluronidase and chondroitinase ABC digestions, followed by separation of the digestion products on Sephadex G-50. In a separate experiment, nitrous acid treatment (16) and chondroitinase ABC digestion, after streptococcal hyaluronidase digestion, degraded all of the $^{35}SO_4$ radioactivity. In addition, an unidentified acetate-labeled component was observed after streptococcal hyaluronidase digestion in the Sephadex elution pattern adjacent to the void volume fraction. This component accounted for about 25% of the total counts from the control cultures and 18% from the stimulated cells in these experiments. Numbers in parentheses refer to the per cent composition.

* Results are expressed as cpm/10^6 cells.

simian virus 40-transformed normal human fibroblasts in the presence and absence of puromycin has also been observed (J. Hopwood and A. Dorfman, unpublished results).

DISCUSSION

The biosynthesis of proteoglycans is thought to follow a pattern in which formation of the protein core is followed by stepwise addition of monosaccharide units catalyzed by the sequential action of six distinct glycosyltransferases and appropriate sulfotransferases (21). There are a number of possible sites at which this scheme may be interrupted, all resulting in a reduced content of chondroitin sulfate proteoglycan. Puromycin, an inhibitor of protein synthesis, has been shown to reduce glycosaminoglycan production, presumably by preventing synthesis of the protein core (22). In two studies, puromycin-induced inhibition of chondroitin sulfate synthesis in cartilaginous tissue was overcome by the addition of D-xylose (7) and *p*-nitrophenyl-β-D-xyloside (9).

Treatment with BrdU has been shown to reduce chondroitin sulfate production in cultured limb bud cells (12) and chondrocytes (18–20), by an unknown mechanism. However, progeny of BrdU-treated mesenchyme cells, which normally exhibit a marked reduction in synthesis of glycosaminoglycans and BrdU-treated chondrocytes, can both be stimulated to produce higher levels by the addition of D-xylose (8, 23).

The present results confirm and extend these early studies, indicating that *p*-nitrophenyl-β-D-xylopyranoside and 4-methylumbelliferyl-β-D-xylopyranoside even more strikingly increase glycosaminoglycan synthesis in chondrocytes and limb bud cells treated with puromycin, BrdU, or both of these compounds.

Presumably, the β-xylosides act as initiators of chondroitin sulfate chain synthesis by joining the intracellular enzyme machinery at the second glycosyltransfer step. In this way, the need for core protein and xylosyltransferase is eliminated. The reason xylose shows less pronounced effects may be due to several factors. Among these are: (*i*) poorer uptake by the cells, (*ii*) a mixture of α and β anomers, or (*iii*) specificity of galactosyltransferase for a xyloside linkage. In a cell-free system, *p*-nitrophenyl-β-D-xyloside appears to be a substrate for the first galactosyltransferase at a much lower optimal concentration than D-xylose (unpublished results).

The specificity for the β-D-xylosides in this system appears absolute so that *p*-nitrophenyl-α-D-xyloside is not a substrate. Furthermore, other sugar derivatives do not initiate chondroitin sulfate chain synthesis further along in the sequence, i.e., β-D-galactosides and β-D-*N*-acetylgalactosamine. These findings are consistent with the previously reported studies on substrate specificities of the various chondroitin sulfate glycosyltransferases, which showed that only the first galactosyltransferase can use a monosaccharide, D-xylose, as an acceptor.

β-D-Xylosides can exert the stimulatory effect even when protein synthesis is inhibited by greater than 95% in all the cells tested. This observation indicated that formation of new transferase enzymes is not involved in increased glycosaminoglycan synthesis induced by the β-D-xylosides. Furthermore, since both puromycin- and BrdU-treated chondrocytes and limb bud cells are capable of being rapidly stimulated (1–2 hr), the cells must contain the necessary precursors and enzymic machinery required for the synthesis of chondroitin sulfate chains. (Under certain conditions of treatment with BrdU, reduced levels of glycosyltransferase activities, and concomitantly lower levels of stimulation by the β-xyloside compounds, have been observed.)

The chondroitin sulfate chains produced by mesenchyme cells stimulated by 4-methylumbelliferyl-β-D-xyloside appear

to be in the same molecular weight range as chondroitin sulfate chains from other cartilage sources. This result implies that the β-xyloside is not only used as a substrate for the first galactosyltransferase reaction, but also induces the formation of complete chondroitin sulfate chains. That the newly synthesized chondroitin sulfate chains produced under the influence of 4-methylumbelliferyl-β-D-xyloside bear the added substrate at the reducing end has been confirmed by obtaining coincident peaks of radioactivity and fluorescence after separation of labeled polysaccharide material by gel chromatography (unpublished results). The chondroitin sulfate chains produced under the influence of the β-xylosides appear to be more soluble than chondroitin sulfate proteoglycan since most of the increase in $^{35}SO_4$ incorporation is observed in material isolated from the medium rather than in that which is cell-associated (unpublished results).

Most interesting in this study is the finding that, whereas untreated chondrocytes and differentiated limb bud cells are stimulated only about 2-fold by the addition of the β-xylosides, noncartilaginous cells and predifferentiated mesenchyme cells, which normally produce low levels of chondroitin sulfate, are stimulated to much higher levels. In these cells an increase in total sulfated glycosaminoglycan production of 3- to 5-fold was generally observed (Tables 4 and 5) with a specific increase of about 10-fold in the synthesis of the chondroitin 4/6-sulfate and dermatan sulfate fraction (Table 4). These findings suggest that noncartilage cells have a much greater potential for the synthesis of chondroitin sulfate chains than is expressed by their production of chondroitin sulfate proteoglycan under uninduced conditions. The reason these cells contain the biosynthetic components for production of the polysaccharide moiety of this complex molecule is not clear. It may represent a vestige of a function once common to all cells, which has now been lost due to an inability to produce the chondroitin sulfate-specific core protein. Alternatively, but less likely, the glycosyltransferases, which synthesize chondroitin sulfate chains under the stimulatory influence of the β-xylosides, may participate in glycosyltransfer reactions involved in the production of other complex macromolecules in the normal uninduced state.

The results obtained in this investigation demonstrate that β-D-xylosides and D-xylose stimulate the production of chondroitin sulfate chains when supplied exogenously to cartilage-derived cells suppressed in their normal synthesis of chondroitin sulfate proteoglycan. It is suggested, therefore, that these compounds may be used as specific probes for the potential to synthesize chondroitin sulfate chains and, thus, may separate the cellular capacity to produce polysaccharide chains and xylosylated core protein.

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